Patterns of Direct Projections from the Hippocampus to the Medial Septum-Diagonal Band Complex

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PATTERNS OF DIRECT PROJECTIONS FROM THE HIPPOCAMPUS TO THE MEDIAL SEPTUM–DIAGONAL BAND COMPLEX: ANTEROGRADE TRACING WITH *PHASEOLUS VULGARIS* LEUCOAGGLUTININ COMBINED WITH IMMUNOHISTOCHEMISTRY OF CHOLINE ACETYLTRANSFERASE

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Abstract—The projections from the Ammon's horn to the cholinergic cell groups in the medial septal and diagonal band nuclei were investigated with anterograde tracing of *Phaseolus vulgaris* leucoagglutinin combined with immunocytochemical detection of choline acetyltransferase, in the rat. Tracer injections were placed into various fields of the septal and temporal parts of the Ammon's horn (CA1–3). These injections revealed differential distributions of *Phaseolus vulgaris* leucoagglutinin-labeled projections in both the lateral septal area and the medial septum–diagonal band complex. In addition to the labeling of dense axonal networks in the lateral septal area, significant numbers of arborizing fibers were labeled in the medial septal and diagonal band nuclei, in particular after tracer injections into the fields CA2–3. The distributions of the projections to the medial septum–diagonal band complex arising from the septal portion of fields CA1 and CA2–3 are similar. In contrast, the septal part and temporal portion of field CA3 project in a topographically differentiated manner to the medial septum and nuclei of the diagonal band. The septal pole of the Ammon's horn innervates the dorsal and medial parts of the medial septal nucleus and the anterior and dorsal parts of the vertical limb of the diagonal band. Axons of the temporal pole of the hippocampus reach the lateral and ventral parts of the medial septum and the intermediate, caudal and ventral parts of the vertical limb of the diagonal band.

These results demonstrate direct feedback projections of the Ammon's horn to the medial septum–diagonal band complex, which show a topographic organization mainly as a function of the septotemporal level of the hippocampus. Within the medial septal and diagonal band nuclei, the labeled varicosities are formed in close proximity to the cell bodies and dendrites of the cholinergic neurons.

The septal complex is known to be reciprocally connected with the hippocampal formation. A well-characterized hippocampal input originates in the medial septal nucleus (MS) and the nuclei of the diagonal band of Broca (DB), and terminates in all parts of the Ammon's horn and dentate gyrus (DG).[27] This septohippocampal projection provides the afferent cholinergic innervation of the hippocampal formation.[9,10,24,26] Studies combining retrograde tracing with immunocytochemical identification of neurons have demonstrated that only a part of the neurons contributing to the septohippocampal projection is cholinergic.[3,4,31,43] Medial septal neurons containing glutamate decarboxylase were demonstrated to project to the hippocampal formation as well.[19] These GABA-synthesizing neurons form synapses, in particular with GABA-containing hippocampal interneurons.[8]

The reverse hippocamposeptal projection is characterized by a strong innervation of the lateral septal area (LS). The neurons in the LS in turn intensely project to the MS and DB.[25,30,37,38] It was demonstrated that a major population of lateral septal neurons utilize GABA as a neurotransmitter,[18,28,29] and are responsible for the major inhibitory, GABAergic synaptic input to MS–DB cholinergic neurons.[20]

A number of tracing studies in various species, however, have provided increasing evidence that, next to the indirect pathway via the lateral septum, hippocampal neurons also directly project to the medial septum and diagonal band nuclei.[1,2,7,15] By retrograde tracing in the rat brain, hippocampal neurons projecting to the MS and DB have been identified as mainly non-pyramidal cells, whereas those sending axons to the LS appeared to be exclu-

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Abbreviations: CA1–3, fields 1–3 of the Ammon's horn; ChAT, choline acetyltransferase; DAB, diaminobenzidine; DB, nuclei of the diagonal band of Broca; DG, dentate gyrus; HDB, nucleus of the horizontal limb of the diagonal band of Broca; LS, lateral septal nucleus; MS, medial septal nucleus; PAP, peroxidase–antiperoxidase; PB, phosphate buffer, PBS-T, phosphate-buffered saline with Triton; PHA-L, *Phaseolus vulgaris* leucoagglutinin; VDB, nucleus of the vertical limb of the diagonal band of Broca.

sively pyramidal neurons.[2] The non-pyramidal hippocamposeptal neurons have been traced in all fields of the Ammon's horn and in the hilus.[2,32] A detailed characterization of the hippocamposeptal projections to the cholinergic nuclei of the MS–DB complex, however, has not previously been undertaken.

In this investigation, we therefore studied the hippocamposeptal innervation of the MS and DB by anterograde tracing with *Phaseolus vulgaris* leucoagglutinin (PHA-L).[13] PHA-L injections were placed in restricted parts of the Ammon's horn and dentate gyrus of the dorsal and ventral halves of the hippocampal formation in order to establish the terminal field of the hippocampal subdivisions in the septal area. PHA-L tracing was combined with choline acetyltransferase (ChAT) immunocytochemistry to relate the distribution of the hippocampal afferents with the cholinergic cell population in the MS and DB.

EXPERIMENTAL PROCEDURES

Thirty-three young adult male Wistar rats (Department of Animal Physiology, University of Groningen) weighing 300 g were used in this study. Under deep anesthesia (sodium pentobarbital, i.p., 30 mg/kg body wt and Hypnorm, Duphar, i.m., 0.4 mg/kg body wt), each rat received a single injection of PHA-L into one of the various loci of the dorsal ($n = 29$) or ventral ($n = 4$) hippocampus according to a procedure described in previous reports.[11,12,21] For this purpose, the rat was mounted in a stereotaxic apparatus (Narishige). PHA-L (Vector Labs, U.S.A.; 25 μg/μl), dissolved in 50 mM Tris-buffered saline, pH 7.4, was iontophoretically delivered through glass micropipettes (tip diameter 10–20 μm), using a positive pulsed 6 μA d.c. (7 s on, 7 s off) for 30 min. One week after surgery, the animals were reanesthetized and perfused transcardially with physiological saline followed immediately by 500 ml of a mixture of 4% freshly depolymerized paraformaldehyde, 0.05% glutaraldehyde and 15% saturated picric acid in 0.1 M phosphate buffer (PB), pH 7.4, at 4°C. Following dissection, the brains were stored in PB containing 30% sucrose for dehydration, and subsequently deeply frozen in dry ice and sectioned at a thickness of 30 μm on a cryostat microtome. Free floating sections were processed for (1) single PHA-L staining or (2) simultaneous peroxidase-immunohistochemical detection of ChAT and PHA-L.

Immunohistochemical procedures

Immunolabeling of PHA-L was performed with a rabbit antiserum against PHA-L (rabbit anti-PHA-L, Dakopatts). For detection of ChAT, a goat polyclonal antiserum raised against human placental ChAT was used.[6] All antisera, including the bridging antibodies, were diluted in 10 mM phosphate-buffered saline containing 0.5% Triton X-100 (PBS-T). All incubations were done at 4°C under gentle agitation. Before, after, and in between each incubation, the sections were rinsed at least three times for 10 min with PBS.

Single Phaseolus vulgaris *leucoagglutinin labeling*. Sections were subsequently incubated in the following antisera: rabbit anti-PHA-L (1:2000, 48 h at 4°C), goat anti-rabbit IgG (Sigma, 1:50, 2 h at room temperature), and rabbit peroxidase–antiperoxidase (rabbit PAP, Dakopatts, 1:500). Following the last incubation, the sections were rinsed three times in 50 mM Tris–HCl buffer, pH 7.6, and reacted for 20–60 min with a solution of 0.04% 3,3′-diaminobenzidine tetrahydrochloride (DAB, Sigma) and 0.015% H_2O_2 in 50 mM Tris–HCl, pH 7.6. The reaction was terminated by several rinses in Tris–HCl buffer. The sections were mounted on glass slides, dehydrated through ethanol and xylol, and coverslipped.

Double labeling of Phaseolus vulgaris *leucoagglutinin and choline acetyltransferase*. Prior to the incubation in the primary antisera, the sections were exposed to 10% normal swine serum. The sections were then incubated in a cocktail of rabbit anti-PHA-L (1:5000) and goat anti-ChAT (1:1000) for 48–72 h at 4°C, followed by incubation in swine anti-rabbit IgG (Nordic, 1:50) together with swine anti-goat IgG (Tago, 1:50) for 2 h. Before use, these secondary antisera were preabsorbed on acetone-extracted brain powder (10 mg/ml) for 1 h or, when diluted in PBS-T, on spare

Abbreviations used in the figures

ac	anterior commissure
CA1–3	Ammon's horn, subfields 1–3
cc	corpus callosum
df	dorsal fornix
DG	dentate gyrus
EC	entorhinal cortex
f	fornix
fi	fimbria
Gr	granular layer
HDB	nucleus of the horizontal limb of the diagonal band of Broca
Hil	hilus
LS	lateral septum
LV	lateral ventricle
Mol	molecular layer of the dentate gyrus
MS	medial septum
Or	stratum oriens
ox	optic chiasm
Pyr	stratum pyramidale
Rad	stratum radiale
SFi	septofimbrial nucleus
sm	stria medullaris of the thalamus
TS	triangular septal nucleus
Tu	olfactory tubercle
VDB	nucleus of the vertical limb of the diagonal band of Broca
vhc	ventral hippocampal commissure

Fig. 1. (A)–(D) Photomicrographs of PHA-L injection sites in fields CA1 (A) and CA3 (B, C) of the dorsal hippocampus, and in field CA3 of the ventral hippocampus (D, horizontal section). In C, additional neurons are labeled in the hilus (Hil) and the granular layer (Gr) of the DG. (E), (F) Low- (E) and high-power (F) photomicrographs illustrating the PHA-L-immunolabeled fibers innervating the lateral septal complex (LS). (E) Innervation of the dorsal quadrant of the LS by field CA3 neurons in the dorsal hippocampus. (F) Close-up of a labeled fiber network in the ventral part of the LS following injection of PHA-L in field CA3 of the temporal hippocampus. (G), (H) Photomicrographs of PHA-L/ChAT double labeling illustrating dorsal and ventral hippocampal efferents innervating the dorsomedial (G) and ventrolateral (H) parts of the medial septum, respectively. The black stained beaded fibers and terminals can easily be distinguished from the brown stained ChAT-immunopositive cell bodies (thick arrows in G and H) in the original material. Scale bars = 500 μm (A, B); 100 μm (C–E); 50 μm (F–H).

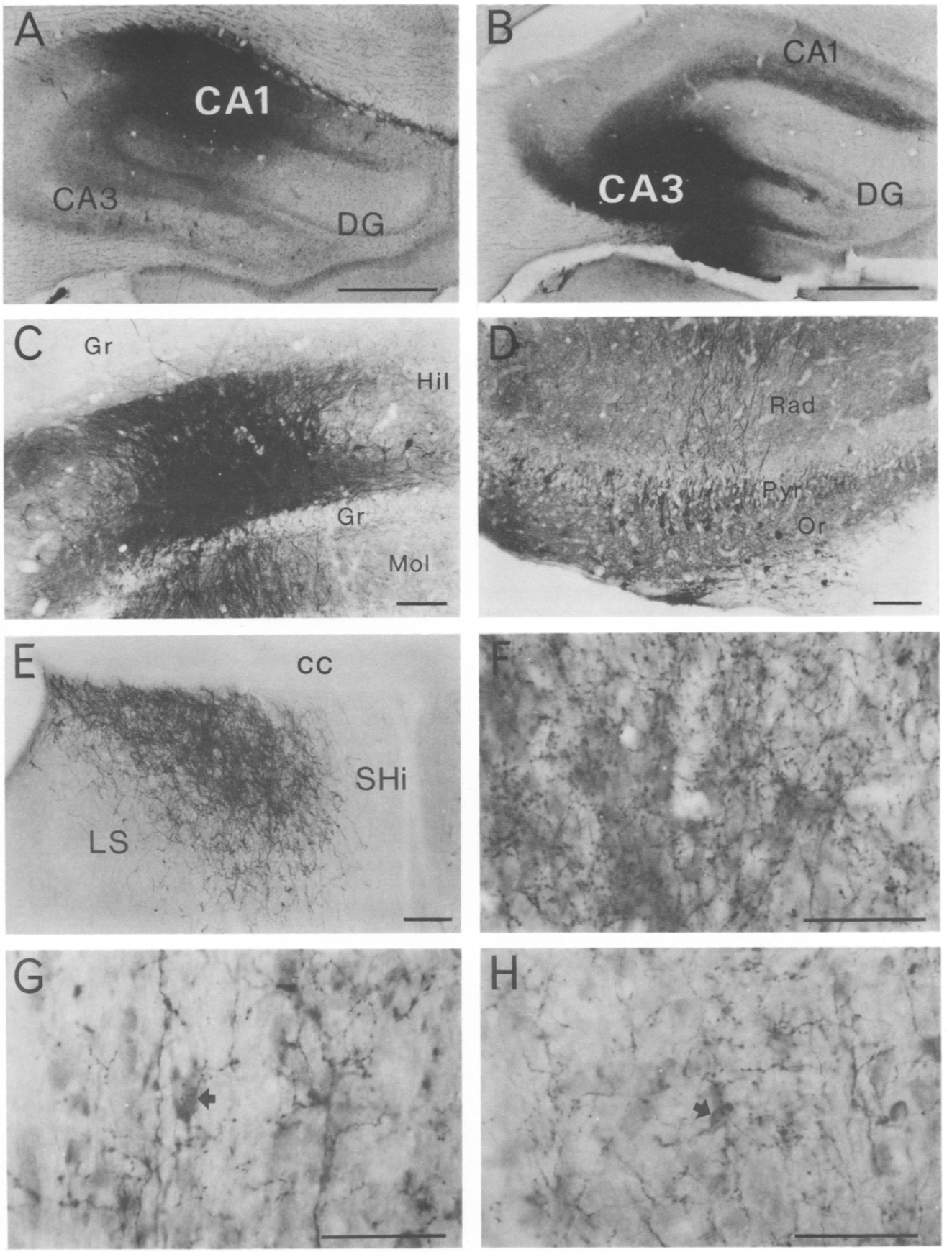

Fig. 1.

sections for 20 h. The sections were then incubated in rabbit PAP (Dakopatts, 1:500) for 2 h and reacted with nickel-enhanced DAB (15 mg DAB and 200 mg nickel ammonium sulfate diluted in Tris–HCl, pH 8.0, and subsequently 0.01% H_2O_2 added) to reveal black PHA-L-labeled fibers.[22] Thereafter ChAT was visualized by incubation of the tissue in goat PAP (Dakopatts, 1:500) and development in DAB (0.04% in Tris–HCl, pH 7.6) and H_2O_2 (0.01%). There appeared to be no non-specific cross-reactivity, since omitting one of the two primary or secondary antibodies resulted in total absence of staining of one of the two antigens. The background staining was substantially reduced by preincubation of the sections in normal swine serum and by preabsorption of the secondary antibodies on acetone-extracted brain powder or on free floating spare sections.

RESULTS

Nomenclature

The terminology of the areal subdivision of the hippocampus and septal area used in the present study is derived from the review written by Swanson *et al.*[39] The nomenclature of the diagonal band nuclei used here is largely based on the parcellation used by Záborsky *et al.*,[45] and is explained in more detail in a previous study.[11] Briefly, the angular portion of the DB is referred to as the ventral part of the vertical limb of the diagonal band of Broca. The aggregation of neurons that we refer to as the nucleus of the horizontal limb of the diagonal band of Broca appears rostrally at the level of the crossing of the anterior commissure and extends caudally for about a millimeter, comprising the area which is also referred to as the magnocellular preoptic nucleus.

Survey of the experiments

The injection sites were defined as the zone containing dark reaction product in which densely stained cell bodies could be discerned. In general, both pyramidal and non-pyramidal neurons were labeled after injections in the Ammon's horn. The injection sites were measured to be 250–1000 μm in diameter, of which some representative examples are shown in Fig. 1A–D. In total, 25 injections successfully labeled neurons in various parts of the Ammon's horn. Of the 23 dorsally placed injections, four were confined to field CA1 (Fig. 1A), four exclusively involved neurons of field CA3, two labeled cells in fields CA1 and CA2, and two involved both fields CA2 and CA3. Two injections were confined to dentate granule cells and one involved the hilus and granule cells of the DG. Eight injections labeled cells in both parts of the Ammon's horn and the DG [CA1 and DG: 2; CA3 and DG: 3 (Fig. 1B, C); parts of all fields CA1–3 and DG: 3]. Two injections revealed strong PHA-L immunoreactivity of field CA3 cells in the temporal part of the hippocampus (Fig. 1D).

General innervation patterns of the septal area

All experiments exhibiting PHA-L-labeled neurons in the Ammon's horn revealed labeling of characteristic, dense intrinsic innervation patterns of the (retro-)hippocampal formation and extrinsic projections to the lateral and posterior septal areas consistent with the present knowledge of hippocampal connectivity. The projections into these septal areas arising from fields CA1–3 are highly ordered in a topographic fashion related to the particular field and the septotemporal position of the injection into the hippocampus. The observed patterns of axonal labeling in the fimbria–fornix system and terminal labeling in the lateral and posterior septal regions are in full agreement with the findings of Swanson and Cowan,[37] and will not be described here in detail. Since in the study of Swanson and Cowan[37] anterograde tracing was performed with tritiated amino acids, no distinction could be made between axons of passage or terminating fibers in the autoradiograms. Our results clearly demonstrate that both lateral septal and septofimbrial nuclei receive dense terminal networks of thin beaded fibers (Figs 1E, F and 2B, C, E, F). In contrast, the triangular septal nucleus only receives sparse terminating fibers, whereas the majority of labeling concerns axons of passage (Fig. 2B, E). Following injections into fields CA2–3, a small number of labeled axons could be followed entering the postcommissural fornix and reaching the posterior hypothalamic area. No septal projection was observed following injections confined to dentate granule cells.

The most striking observation on the PHA-L-labeled projections of the Ammon's horn was the invasion of a large number of fibers into the MS after crossing the lateral septal area. These fibers course ventrally through the MS and subsequently invade the vertical and horizontal limbs of the diagonal band of Broca (VDB and HDB). Along their way through the MS–DB complex, the fibers branch frequently and contain numerous "*en passant*" and terminal boutons (Fig. 1G, H), which provides clear evidence that the Ammon's horn directly projects to the MS, VDB, and HDB.

Innervation of medial septum–diagonal band complex by hippocampal fibers in relation to the cholinergic cells

Sections which were double-stained for PHA-L and ChAT showed the pattern of the hippocampal innervation in relation to the exact position of the cholinergic neurons and dendritic processes in the MS and the VDB, and HDB. A continuous cluster of reddish-brown stained ChAT-immunoreactive perikarya was present in the MS, VDB, and HDB (see quadrangular dots in Figs 3 and 4). The distribution of these ChAT-immunoreactive neurons is concordant with previous studies based on immunolabeling of ChAT.[41] All injections in the septal portion of the hippocampus, which involved the Ammon's horn regions 1–3, gave rise to black-colored, labeled fibers terminating in the cholinergic nuclei of the MS–DB complex (Figs 1G and 3). These injections in fields

CA1–3 of the septal portion of the hippocampus revealed similar termination patterns in the MS, VDB, and medial portion of the HDB. Nevertheless, fields CA2 and CA3 provide a considerably larger number of axons and endings in these nuclei than does CA1. Moreover, the sparse projection from field CA1 is mainly ipsilateral, whereas the efferents of fields CA2 and CA3 are distributed in the MS, VDB, and HDB in a bilateral, symmetric fashion, although the ipsilateral projection is slightly more extensive (Fig. 3B–D). One experiment with PHA-L deposit mainly in the hilar region of the DG yielded only few labeled fibers in the medial septum.

As is the case in the lateral septal complex, the distribution of the terminal labeling in the MS–DB complex arising from the septal portion of field CA3 shows a different topography, almost complementary to the efferents arising from the temporal portion of

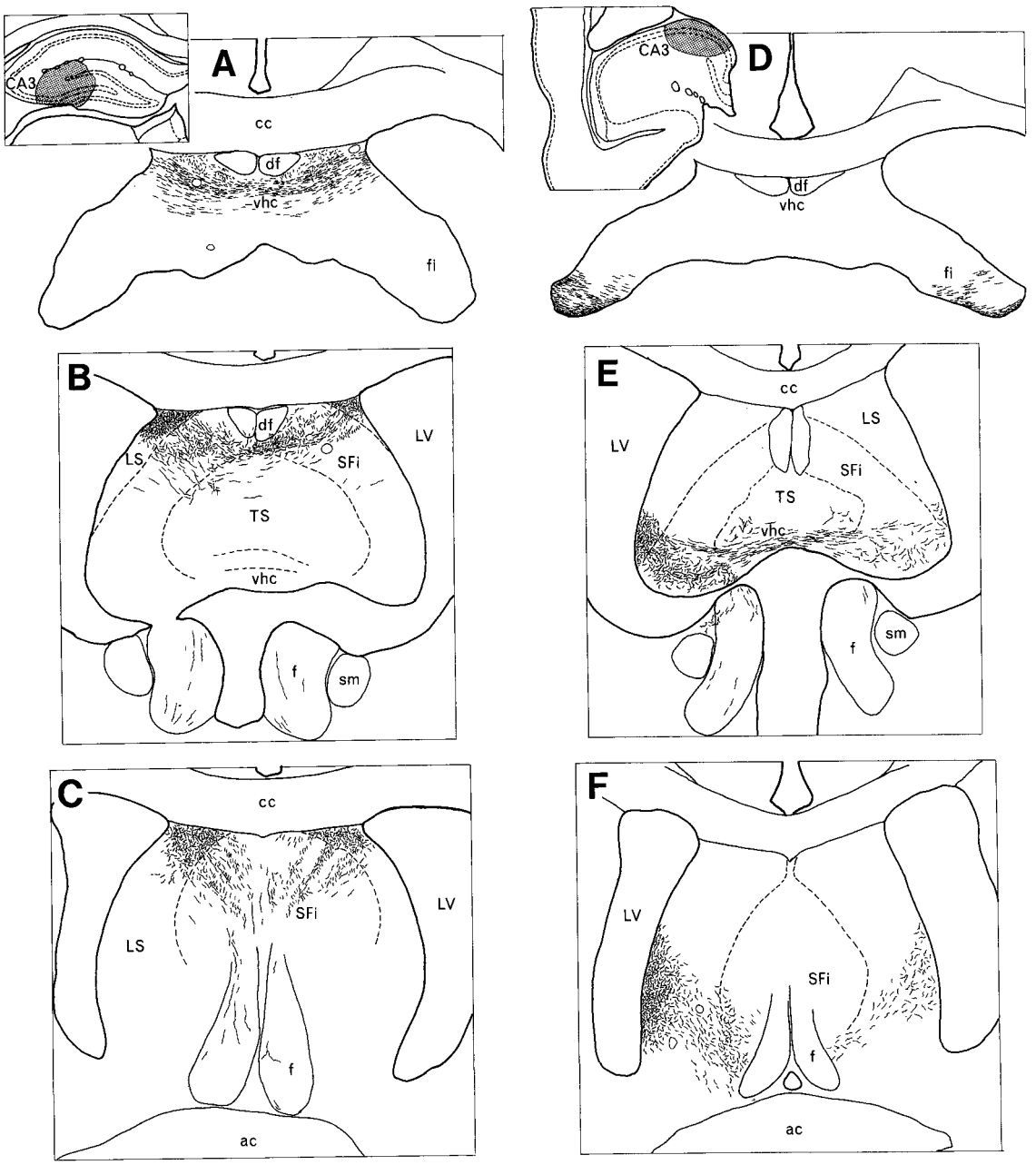

Fig. 2. Chartings of two series of transverse sections of the rat fimbria–fornix system (A, D) and the caudal aspects of the septal area (B, C, E, F) illustrating the axon trajectories and projections to the lateral septal, septofimbrial and triangular septal nuclei. The left (A–C) and right (D–F) columns show the innervation patterns after injections in the septal and temporal parts of field CA3, respectively. The injection sites are depicted in the inserts at the left of each column, and are identical to those illustrated in Fig. 1B and D, respectively. In D, the insert represents a horizontal section through the ventral hippocampal region.

field CA3 (compare Fig. 3 with Fig. 4). This differentiated topographic arrangement of the projections from the septal versus temporal Ammon's horn is most pronounced in the MS and the anterior portion of the VDB.

After injection of PHA-L in the septal part of field CA2–3, labeled fibers approach the MS from a dorsal direction (Fig. 3B). They run ventrally through, particularly, the dorsal and medial parts of the MS on both sides of the midline, and encroach on the medial portion of the VDB at the level where the cholinergic neurons of the VDB form an uninterrupted continuity with those of the MS (Fig. 3C). Here the fibers turn in a rostral and laterocaudal direction to reach the whole rostrocaudal extent of the VDB, as defined by the distribution of the cell bodies of ChAT-

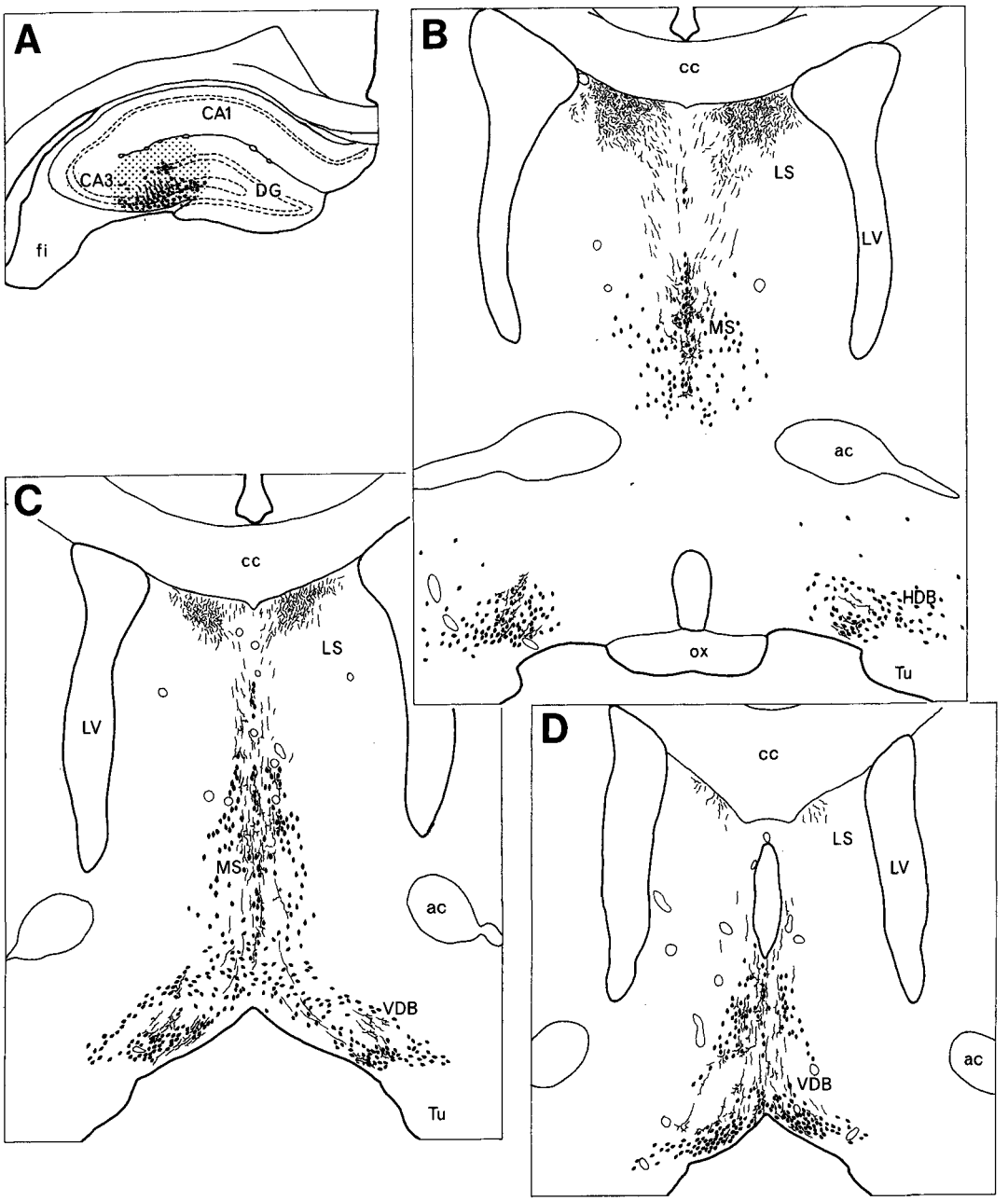

Fig. 3. Series of drawings of transverse sections through the rostral parts of the rat septal area (B–D) showing the anterogradely labeled projections in the lateral and medial septum and the vertical and horizontal limbs of the diagonal band of Broca, following a PHA-L injection in field CA3 of the dorsal hippocampus (A). The projections are drawn together with the ChAT-immunopositive cell bodies in the MS, VDB, and HDB, which are plotted as neuron-shaped dots. The chartings represent the same experiment as those in Fig. 2A–C.

positive magnocellular neurons (Fig. 3C, D). In the angular wings of the VDB, the fibers are slightly more numerous in the cholinergic-cell-poor central core than among the ChAT-immunoreactive somata in the ventral surface of the VDB (Fig. 3C). A small number of labeled projections were observed caudally to innervate the medial portion of the HDB.

Following deposition of PHA-L into the temporal portion of the CA3, a large amount of labeled fibers run medially through the ventral part of the lateral septal complex into the lateral and ventral quadrants of the MS (Fig. 4B), giving off abundant beaded and terminal specializations (Fig. 1H). In contrast to the almost symmetrical, bilateral innervation originating in the septal part of field CA3, the afferents from the temporal portion of field CA3 are mainly aimed at the ipsilateral hemisphere, thus sparsely providing the opposite septal area (Fig. 4B, C). The innervation plexus in the MS continues in the transition zone connecting the MS to the VDB that is rich in cholinergic cells (Fig. 4C). In both rostral and

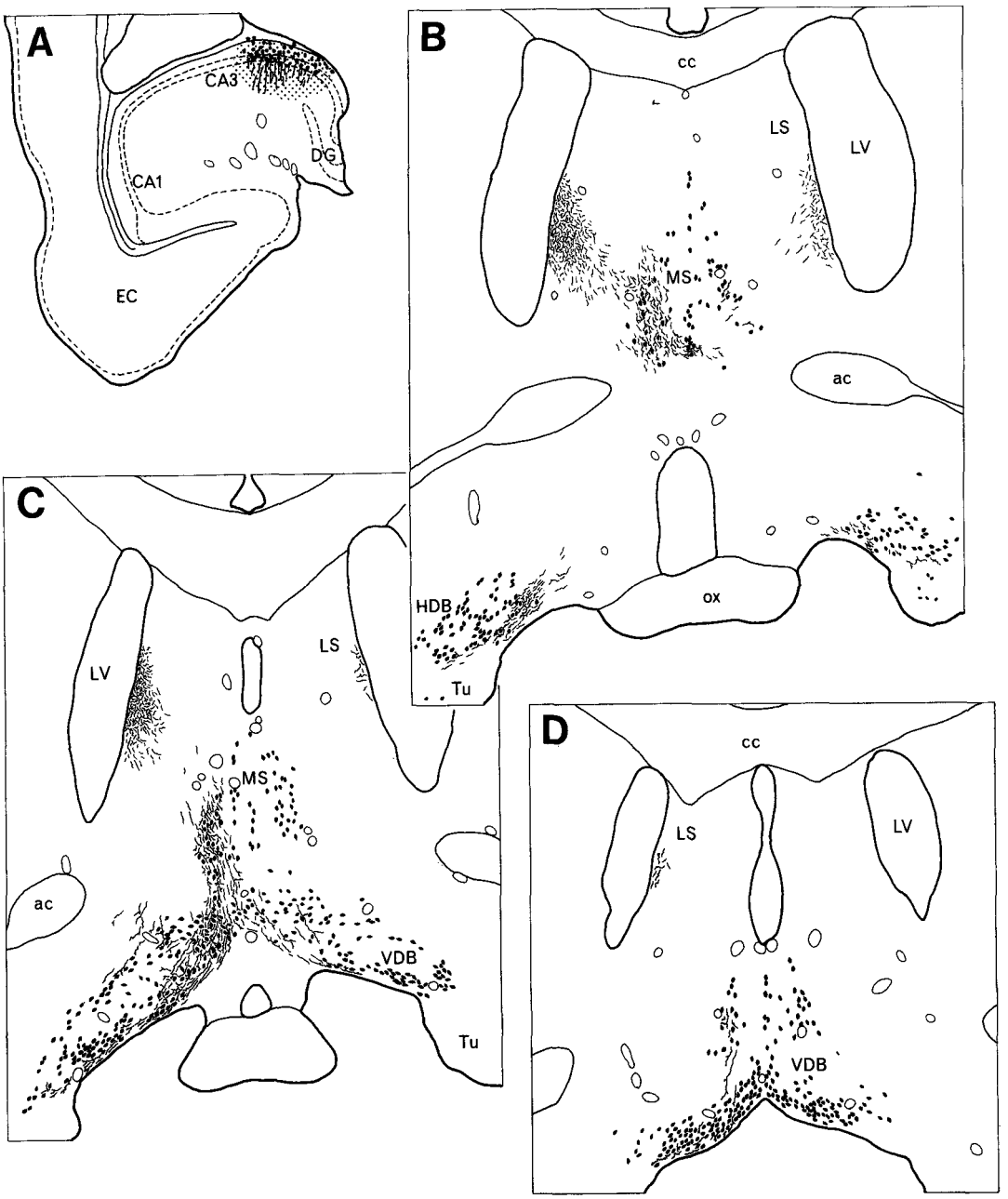

Fig. 4. Distribution of PHA-L-labeled fibers and ChAT-immunoreactive perikarya in the septal and diagonal band nuclei (B–D) following a PHA-L deposit in field CA3 of the ventral hippocampus (A, horizontal section). The chartings represent the same experiment as those in Fig. 2D–F.

caudolateral directions, the fibers follow the contingents of ChAT-immunoreactive neurons of the VDB, among which they richly branch and show numerous terminal endings (Fig. 4B, D). In contrast to the dorsal hippocampal afferents to the diagonal band area, only a few fibers course through the cholinergic-cell-poor central core of the angular wing of the VDB. At more caudal levels, labeled fibers innervate the most medial extension of the HDB, as is the case for the dorsal hippocampal afferents. In addition, the afferent fibers from the temporal hippocampus continue further caudally to form a distinct termination network just dorsal to the supraoptic nucleus of the hypothalamus.

Within the MS and throughout the VDB and medial portion of the HDB, labeled hippocampal fibers give rise to many branches, richly provided with "*en passant*" and terminal specializations (Fig. 1G, H). These specializations are made in close proximity with ChAT-positive cell bodies and dendritic profiles (Fig. 5). These appositions were most frequently observed in the VDB, where the large ChAT-positive neurons display long, immunoreative dendritic arborizations (Fig. 5A, C, E). The afferent fibers show both single (Fig. 5C, E, F) and multiple apposing structures (Fig. 5B, D, G) with ChAT-immunoreactive elements.

DISCUSSION

It has previously been shown that hippocampal neurons send axons not only to the lateral septum, but to the medial septum as well.[2,37] The present experiments have extended these findings by demonstrating that, next to dense terminal fiber networks in the lateral and posterior septal areas, the hippocampus gives rise to less, but still rich projections in the medial septum and diagonal band nuclei as well. From the distribution of projections of the particular fields of the dorsal part of the hippocampus, it can be concluded that the strongest innervation of the MS–DB complex arises from fields CA2–3 of the Ammon's horn. Field CA1 and the hilus of the DG have a relatively minor contribution to the input to the MS and DB nuclei.

The dorsal/septal and ventral/temporal parts of field CA3 provide efferents terminating within the MS–DB complex in a complementary, topographic pattern, as in the lateral septal area (Fig. 6). A striking difference, however, between the septal projections of the dorsal and those of the ventral hippocampus is that the dorsally originating CA3 efferents are bilaterally distributed, whereas the ventrally originating projections have a strong preference for the ipsilateral half of the septal and diagonal band nuclei.

With respect to the intralamellar topography of the hippocamposeptal projection, these findings agree with the results obtained by Alonso and Köhler,[2] who observed the highest number of retrogradely labeled hippocampal neurons in subfield CA3 after horseradish peroxidase injections in the medial septum. However, due to the medially placed injections in the MS, these authors were not able to demonstrate the bilateral distribution of the afferents in the MS which originate in the dorsal portion of field CA3, as shown in this study. Our results, furthermore, show that the hippocampal terminal field extends from the MS dorsally to the most anterior pole of the VDB rostrally, and to the medial aspects of the caudal HDB caudoventrally. This way the hippocamposeptal projection comprises essentially all parts of the basal forebrain cholinergic nuclei that in turn give rise to projections to the hippocampal formation.[3,11,27] In this respect, it can be concluded that the anatomical relationship between the hippocampal formation and the MS–DB cholinergic nuclei is characterized by reciprocal connections. Such a reciprocal relationship has also been demonstrated beween the cholinergic nuclei in the basal forebrain and the prefrontal cortex.[12]

Varicosities seen in the light microscope of the PHA-L-labeled hippocampal fibers represent synaptic terminals.[22,44] Appositions of PHA-L-labeled presynaptic specializations of the projections from the Ammon's horn with ChAT-immunoreactive cell bodies and dendrites in the MS–DB complex indicate a direct feedback input to the cholinergic projection to the hippocampus. Electron microscopic verification, however, remains a prerequisite to substantiate the monosynaptic nature of the hippocampal fibers upon the MS–DB cholinergic neurons. Recently, Léránth and Frotscher[20] studied the ultrastructure of the hippocampal projections to the cholinergic and GABAergic neurons in the septal area by using anterograde degeneration following fimbria–fornix transection in combination with immunocytochemistry. However, they did not observe degenerated boutons making synapses with ChAT-positive neuronal elements in the medial septum–diagonal band complex. The direct visualization of the hippocamposeptal fibers filled with PHA-L, as shown in our work, may be advantageous in tracing putative synaptic contacts of the hippocampal efferents with MS–DB cholinergic neurons. At any rate, synaptic contacts with MS–DB neuronal elements are more sparse in number in comparison with the hippocampal terminations on the lateral septal neurons, as is also shown in Léránth and Frotscher's study.[20]

Non-cholinergic, largely GABAergic septohippocampal neurons,[8,19] which were not visualized in this study, make up a large part of the MS–DB complex. The GABA-containing cells, also characterized by the presence of parvalbumin, are particularly distributed in the cholinergic cell-poor middle area of the MS and the central portion of the angular wing of the diagonal band area,[17] indicated as the caudal part of the VDB in this study. These latter areas receive hippocampal efferents as well—as shown in this study. The GABAergic neurons projecting to the

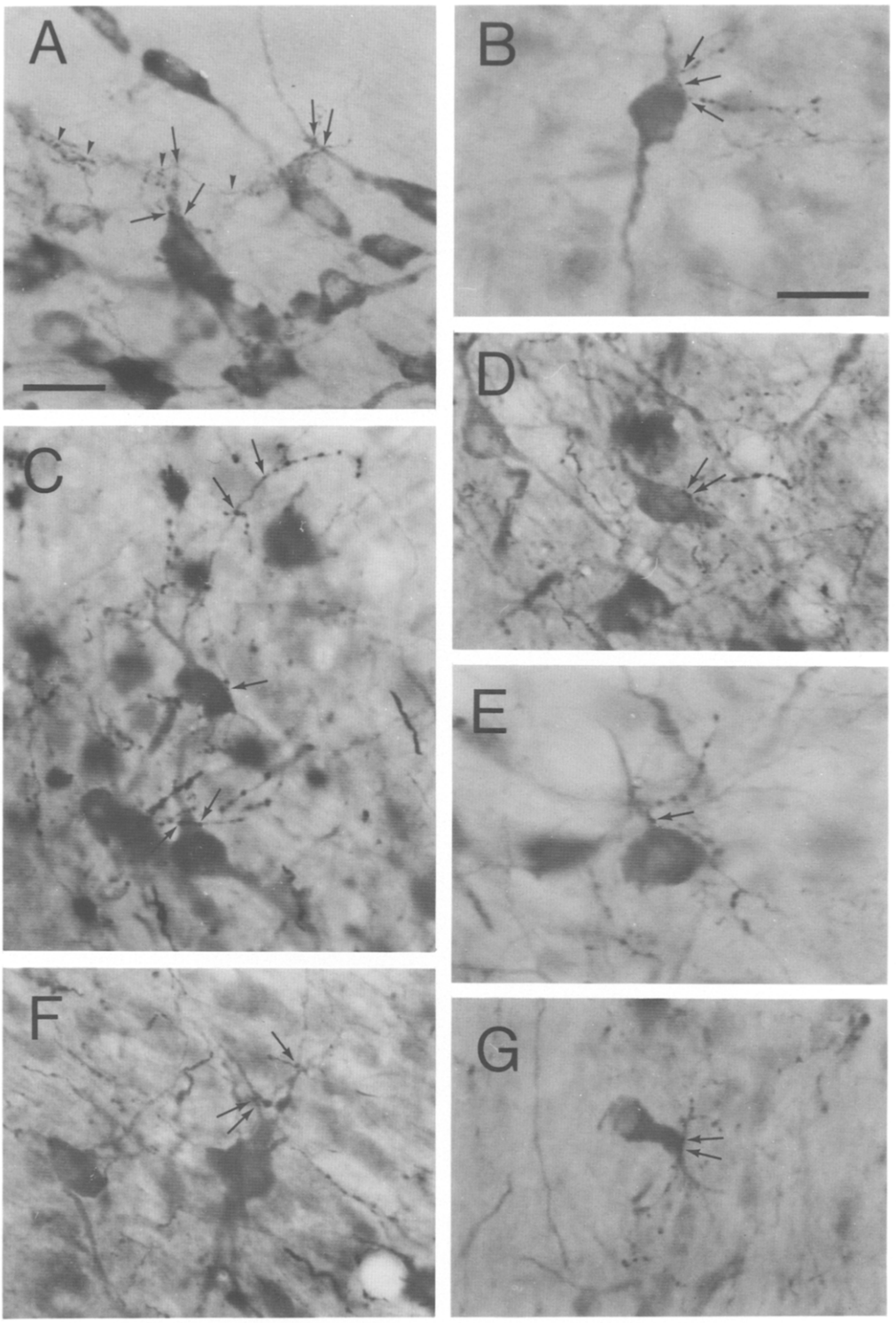

Fig. 5. High-power photomicrographs of PHA-L-labeled projections contacting ChAT-immunoreactive structures in the diagonal band nuclei (A–F) and the medial septum (G). Close appositions between PHA-L-labeled boutons and ChAT-positive profiles are indicated with arrows. In the original material, the bluish black staining of the hippocampal fibers contrasts the brown staining of the ChAT immunolabeling. Both contacts with cell bodies (B, D, E, G) and dendrites (A, C, F) were encountered. In A, the courses of individual fibers are indicated with small arrowheads. Scale bars = 25 μm. The enlargements of B–G are identical.

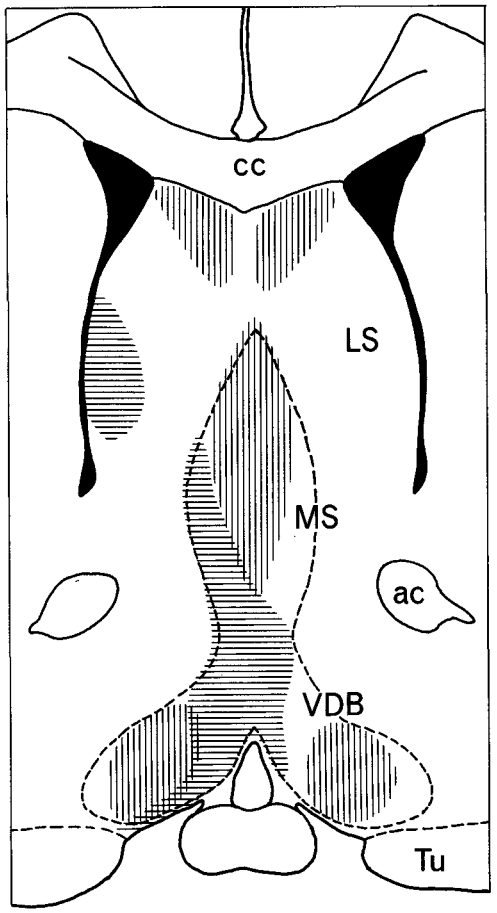

Fig. 6. A diagrammatic survey of the topographic arrangement of the projections to the septal and diagonal band nuclei by field CA3 of the hippocampus. The termination areas of the septal and temporal portions of field CA3 are marked by vertical and horizontal lines, respectively. Notice the bilateral nature of the projection of the septal CA3 versus the strong ipsilateral preference of the projection of the temporal CA3.

hippocampus might thus also be subject to direct feedback control.

Previous studies using retrograde tracing demonstrated that the hippocampal fibers terminating in the MS and DB largely represent collaterals arising from the non-pyramidal neurons in Ammon's horn and hilus, which are heterogeneous in shape and are generally considered as interneurons.[2,32] At present, it is not known to what extent the non-pyramidal, extrinsically projecting and the local-circuit neurons belong to the same or to different populations of cells.[33,40] A part of the MS-projecting cells was demonstrated to contain GABA.[34] The source of the projections to the medial septum might contain cholecystokinin, since hippocampal neurons containing cholecystokinin give rise to axons running into the septum.[14] In contrast to the projection to the medial septum that particularly originates from the non-pyramidal cells, the dense innervation of the lateral septum exclusively arises from the pyramidal neurons of the Ammon's horn.[2] There is considerable biochemical and pharmacological evidence that this pyramidal feedback to the lateral septum is glutamatergic,[16,35,46] and excites the GABAergic neurons in the lateral septum.[23] Nevertheless, it cannot be ruled out that hippocampal pyramidal cells contribute to the innervation of the MS–DB complex as well, although their contribution has been considered to be minor.[2] The existence of a minor excitatory pathway from the hippocampus to the anterior diagonal band area has already been indicated by slight reductions of glutamate content in that part of the DB after fimbria–fornix transection.[42]

CONCLUSION

The relatively strong and topographically organized, direct hippocampal feedback projection to the MS–DB complex, as shown by this investigation, provides new insight into the organization of the hippocampal and septal connectivities. Through direct innervation of the MS–DB complex and thus without interference of the lateral septum, the hippocampus may influence the activity of neurons in the medial septal and diagonal band nuclei, a number of which provide cholinergic and GABAergic input to the hippocampus. The functional significance of this reciprocity for the septohippocampal drive, however, is unclear. It is well known that the septohippocampal projection plays an essential role in the hippocampal rhythmic slow activity (theta rhythm).[5] Recently, it has been demonstrated that the atropine-sensitive, cholinergically induced rhythmic slow activity is dependent on a completely intact septal circuitry, including lateral septal projection upon the MS–DB cholinergic neurons.[36] In contrast to this indirect hippocamposeptal feedback control, the contribution of the direct hippocampal MS–DB projection to this electrophysiological phenomenon remains to be elucidated.

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